CLAIMS

WHAT IS CLAIMED IS:

1. A method for synthesis of a glycoprotein, the method comprising:

a) incorporating into a protein an unnatural amino acid that comprises a first reactive group; and,

b) contacting the protein with a saccharide moiety that comprises a second reactive group, wherein the first reactive group reacts with the second reactive group to attach the saccharide moiety to the unnatural amino acid.

2. The method of claim 1, wherein the first reactive group is an electrophilic moiety and the second reactive group is a nucleophilic moiety.

3. The method of claim 2, wherein the electrophilic moiety is a keto or aldehyde moiety.

4. The method of claim 2, wherein the nucleophilic moiety is selected from the group consisting of: —NR^1—NH_2 (hydrazide), —NR^1(C=O)NR^2NH_2 (semicarbazide), —NR^1(C=S)NR^2NH_2 (thiosemicarbazide), —(C=O)NR^1NH_2 (carbonylhydrazide), —(C=S)NR^1NH_2 (thiocarbonylhydrazide), —(SO_2)NR^1NH_2 (sulfonylhydrazide), —NR^1NR^2(C=O)NR^3NH_2 (carbazide), —NR^1NR^2(C=S)NR^3NH_2 (thiocarbazide), and —O—NH_2 (hydroxylamine), where each R^1, R^2, and R^3 is independently H, or alkyl having 1-6 carbons.

5. The method of claim 4, wherein the nucleophilic moiety is selected from the group consisting of hydrazide, hydroxylamine, semicarbazide, and carbohydrazide.

6. The method of claim 2, wherein the reaction product comprises an oxime, an amide, a hydrazone, a carbohydrazone, a thiocarbohydrazone, a sufonylhydrazone, a semicarbazone, or a thiosemicarbazone.

7. The method of claim 6, wherein the reaction product comprises a reduced hydrazone.

8. The method of claim 1, wherein the first reactive group is a nucleophilic moiety and the second reactive group is an electrophilic moiety.

9. The method of claim 8, wherein the electrophilic moiety is a keto or aldehyde moiety.

10. The method of claim 1, wherein the saccharide moiety comprises two or more carbohydrate moieties.

11. The method of claim 1, further comprising: c) contacting the saccharide moiety with a glycosyltransferase, a sugar donor moiety, and other reactants required for glycosyltransferase activity for a sufficient time and under appropriate conditions to transfer a sugar from the sugar donor moiety to the saccharide moiety.

12. The method of claim 11, wherein the glycosyltransferase is selected from the group consisting of: a galactosyltransferase, a fucosyltransferase, a glucosyltransferase, an N-acetylgalactosaminyltransferase, an N-acetylglucosaminyltransferase, a glucuronyltransferase, a sialyltransferase, a mannosyltransferase, a glucuronic acid transferase, a galacturonic acid transferase, and an oligosaccharyltransferase.

13. The method of claim 11, wherein the method further comprises contacting a product of step (c) with at least a second glycosyltransferase and a second sugar donor moiety.

14. The method of claim 11, wherein the saccharide moiety comprises a terminal GlcNAc, the sugar donor moiety is UDP-Gal and the glycosyltransferase is a β-1, 4-galactosyltransferase.

15. The method of claim 11, wherein the saccharide moiety comprises a terminal GlcNAc, the sugar donor moiety is UDP-GlcNAc and the glycosyltransferase is a β1-4N-acetylglucosaminyltransferase.

16. The method of claim 15, wherein the method further comprises contacting the product of the N-acetylglucosaminyltransferase reaction with a β1-4mannosyltransferase and GDP-mannose to form a saccharide moiety that comprises Manβ1-4GlcNAcβ1-4GlcNAc-.

17. The method of claim 16, wherein the method further comprises contacting the Manβ1-4GlcNAcβ1-4GlcNAc- moiety with an α1-3mannosyltransferase and GDP-mannose to form a saccharide moiety that comprises Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc-.

18. The method of claim 17, wherein the method further comprises contacting the Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc- moiety with an α1-6mannosyltransferase and

GDP-mannose to form a saccharide moiety that comprises Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-.

19. The method of claim 18, wherein the method further comprises contacting the Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc- moiety with a β1-2N-acetylglucosaminyltransferase and UDP-GlcNAc to form a saccharide moiety that comprises Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-.

20. The method of claim 19, wherein the method further comprises contacting the Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc- moiety with a β1-2N-acetylglucosaminyltransferase and UDP-GlcNAc to form a saccharide moiety that comprises GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-.

21. The method of claim 11, wherein the method further comprises contacting the saccharide moiety with one or more of a β1-4N-acetylglucosaminyltransferase, an α1,3fucosyltransferase, an α1,2 fucosyltransferase, an α1,4fucosyltransferase, a β1-4galactosyltransferase, and a sialyltransferase, to form a biantennary or triantennary oligosaccharide structure.

22. The method of claim 1, wherein the incorporating step is in vivo.

23. The method of claim 1, wherein the incorporating step comprises using an orthogonal tRNA/orthogonal aminoacyl-tRNA synthetase (O-tRNA/O-RS) pair, wherein the O-tRNA recognizes a selector codon and incorporates the unnatural amino acid into the protein in response to the selector codon, and wherein the O-RS preferentially aminoacylates the O-tRNA with the unnatural amino acid.

24. The method of claim 23, wherein the O-RS comprises an amino acid sequence comprising any one of SEQ ID NO.: 1, 2, or 3.

25. The method of claim 23, wherein the O-tRNA comprises a $\text{mutRNA}^{\text{Tyr}}_{\text{CUA}}$.

26. A glycoprotein produced by the method of claim 1.

27. A glycoprotein produced by the method of claim 22.

28. A glycoprotein comprising a saccharide moiety and a polypeptide, wherein the saccharide moiety is attached to the polypeptide by a reaction product of a nucleophilic reaction between a first reactive group attached to an unnatural amino acid present in the polypeptide and a second reactive group attached to the saccharide moiety.

29. The glycoprotein of claim 28, wherein the first reactive group is an eletrophilic moiety and the second reactive group is a nucleophilic moiety.

30. The glycoprotein of claim 29, wherein the electrophilic moiety is keto or aldehyde moiety.

31. The glycoprotein of claim 29, wherein the nucleophilic moiety is selected from the group consisting of: —NR^1—NH_2 (hydrazide), —NR^1(C=O)NR^2NH_2 (semicarbazide), —NR^1(C=S)NR^2NH_2 (thiosemicarbazide), —(C=O)NR^1NH_2 (carbonylhydrazide), —(C=S)NR^1NH_2 (thiocarbonylhydrazide), —(SO_2)NR^1NH_2 (sulfonylhydrazide), —NR^1NR^2(C=O)NR^3NH_2 (carbazide), —NR^1NR^2(C=S)NR^3NH_2 (thiocarbazide), or —O—NH_2 (hydroxylamine), where each R^1, R^2, and R^3 is independently H, or alkyl having 1-6 carbons.

32. The glycoprotein of claim 31, wherein the nucleophilic moiety is selected from the group consisting of hydrazide, hydroxylamine, semicarbazide, and carbohydrazide.

33. The glycoprotein of claim 28, wherein the reaction product comprises an oxime, an amide, a hydrazone, a carbohydrazone, a thiocarbohydrazone, a sufonylhydrazone, a semicarbazone, or a thiosemicarbazone.

34. The glycoprotein of claim 33, wherein the reaction product comprises a reduced hydrazone.

35. A method for synthesis of a glycoprotein, the method comprising incorporating into a protein an unnatural amino acid that comprises a saccharide moiety.

36. The method of claim 35, wherein the method further comprises contacting the saccharide moiety with a glycosyltransferase, a sugar donor moiety, and other reactants required for glycosyltransferase activity for a sufficient time and under appropriate conditions to transfer a sugar from the sugar donor moiety to the saccharide moiety.

37. The method of claim 36, wherein the glycosyltransferase is selected from the group consisting of: a galactosyltransferase, a fucosyltransferase, a glucosyltransferase, an N-acetylgalactosaminyltransferase, an N-acetylglucosaminyltransferase, a glucuronyltransferase, a sialyltransferase, a mannosyltransferase, a glucuronic acid transferase, a galacturonic acid transferase, and an oligosaccharyltransferase.

38. The method of claim 36, wherein the method further comprises contacting the product of the glycosyltransferase reaction with at least a second glycosyltransferase and a second sugar donor moiety.

39. The method of claim 36, wherein the saccharide moiety comprises a terminal GlcNAc, the sugar donor moiety is UDP-GlcNAc and the glycosyltransferase is a β1-4N-acetylglucosaminyltransferase.

40. The method of claim 36, wherein the saccharide moiety comprises a terminal GlcNAc, the sugar donor moiety is UDP-Gal and the glycosyltransferase is a β1-4-galactosyltransferase.

41. The method of claim 35, wherein the incorporating step comprises using an orthogonal tRNA/orthogonal aminoacyl-tRNA synthetase (O-tRNA/O-RS) pair, wherein the O-tRNA recognizes a selector codon and incorporates the unnatural amino acid into the protein in response to the selector codon, and wherein the O-RS preferentially aminoacylates the O-tRNA with the unnatural amino acid.

42. The method of claim 41, wherein the O-RS comprises an amino acid sequence comprising any one of SEQ ID NO.: 4, 5 or 6.

43. The method of claim 41, wherein the O-tRNA comprises a $\text{mutRNA}^{\text{Tyr}}_{\text{CUA}}$.

44. The method of claim 35, wherein the incorporating step is in vivo.

45. The method of claim 35, wherein the unnatural amino acid comprises a β-O-GlcNAc-L-serine, a tri-acetyl-β-GlcNAc-serine, a tri-O-acetyl-GalNAc-α-threonine, or an α-GalNAc-L-threonine.

46. A glycoprotein produced by the method of claim 35.

47. A host cell for synthesizing a glycoprotein, the host cell comprising:

a) an unnatural amino acid that comprises a saccharide moiety;

b) an orthogonal tRNA that recognizes a selector codon;

c) an orthogonal aminoacyl tRNA synthetase (O-RS) that catalyzes attachment of the unnatural amino acid to the orthogonal tRNA;

d) a polynucleotide that encodes a glycosyltransferase; and

e) a polynucleotide sequence that encodes a polypeptide and comprises at least one selector codon.

48. The host cell of claim 47, wherein the glycosyltransferase is selected from the group consisting of: a galactosyltransferase, a fucosyltransferase, a glucosyltransferase, an N-acetylgalactosaminyltransferase, an N-acetylglucosaminyltransferase, a glucuronyltransferase, a sialyltransferase, a mannosyltransferase, a glucuronic acid transferase, a galacturonic acid transferase, and an oligosaccharyltransferase.

49. The host cell of claim 47, wherein the host cell is a mammalian cell, a yeast cell, a bacterial cell, a plant cell, a fungal cell, an archaebacterial cell, or an insect cell.

50. A composition comprising a translation system, the translation system comprising an orthogonal tRNA (O-tRNA) and an orthogonal aminoacyl tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates the O-tRNA with an unnatural amino acid that comprises a saccharide moiety and the O-tRNA recognizes at least one selector codon.

51. The composition of claim 50, wherein the O-RS comprises an amino acid sequence comprising any one of SEQ ID NO.: 4, 5 or 6, or a conservative variant thereof.

52. The composition of claim 50, wherein the O-RS is encoded by a polynucleotide comprising a polynucleotide sequence of any one of SEQ ID NO.: 8, 9, or 10, or a conservative variant thereof.

53. The composition of claim 50, wherein the O-tRNA comprises a $\text{mutRNA}_{\text{CUA}}^{\text{Tyr}}$.

54. The composition of claim 50, wherein the unnatural amino acid comprises a β-O-GlcNAc-L-serine, a tri-acetyl-β-GlcNAc-serine, a tri-O-acetyl-GalNAc-α-threonine, or an α-GalNAc-L-threonine.

55. An artificial polypeptide selected from the group consisting of:

(a) a polypeptide that comprises an amino acid sequence as shown in any one of SEQ ID NO.: 4-6;

(b) a polypeptide that comprises an amino acid sequence encoded by a polynucleotide sequence as shown in any one of SEQ ID NO.: 8-10;

(c) a polypeptide that is specifically immunoreactive with an antibody specific for a polypeptide of (a), or (b); and,

(d) an amino acid sequence comprising a conservative variation of (a), (b), or (c).

56. An antibody or antisera specifically immunoreactive with the polypeptide of claim 55.

57. An artificial polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a nucleotide sequence as set forth in any one of SEQ ID NO.: 8-10;

(b) a polynucleotide that is complementary to or that encodes a polynucleotide sequence of (a);

(c) a polynucleotide encoding a polypeptide that comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 1-6, or a conservative variation thereof;

(d) a polynucleotide that encodes a polypeptide of claim 55;

(e) a nucleic acid that hybridizes to a polynucleotide of (a), (b), (c), or (d) under highly stringent conditions over substantially the entire length of the nucleic acid;

(f) a polynucleotide that is at least 98% identical to a polynucleotide of (a), (b), (c), (d), or (e); and,

(h) a polynucleotide comprising a conservative variation of (a), (b), (c), (d), (e), or (f).